

5 **WHAT IS CLAIMED IS:**

1. A native, authentic, enzymatically active
NTPase/RNA helicase protein produced by a process
comprising the steps of:

10 a) expressing an NTPase/RNA helicase
 encoding nucleic acid of hepatitis C
 virus in a eukaryotic expression system
 such that a complete, authentic and
 native NTPase/RNA helicase protein is
 synthesized, said authentic and native
15 NTPase/RNA helicase protein comprising
 amino acids 1027 -1657;

 b) extracting NTPase/RNA helicase protein
 from said eukaryotic expression system
 in an enzymatically active form of said
20 protein; and

 c) purifying said NTPase/RNA helicase
 protein such that the enzymatically
 active form of said protein is
 maintained.

25 2. The protein produced according to claim 1,
 said nucleic acid of hepatitis C virus in step a)
 corresponding to a human hepatitis C virus nucleic
 acid.

30 3. The protein produced according to claim 1,
 said nucleic acid of hepatitis C virus in step a) being
 derived from a genotype of the human hepatitis C virus
 nucleic acid.

4. The protein produced according to claim 1,
 wherein said nucleic acid of hepatitis C virus in step

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5 a) is a variant of the human hepatitis C virus.

5. The protein produced according to claim 1,
said nucleic acid of hepatitis C virus in step a)
encoding a complete NS3 coding region.

6. The protein produced according to claim 1,
10 said nucleic acid of hepatitis C virus in step a)
encoding a complete NS3 through NS5B coding region
comprising amino acid residues from 1027 to 3011 of
hepatitis C virus genome.

7. The protein produced according to claim 1,
15 wherein said expression system is a recombinant
baculovirus-insect cell expression system.

8. The protein produced according to claim 1,
wherein the extracted protein is purified by
immunoaffinity chromatography using antibodies specific
20 for hepatitis C virus proteins.

9. The protein produced according to claim 1,
having basal NTPase activity in the range of 0-200 min⁻¹
and RNA helicase activity greater than 0.001 min⁻¹.

10. The protein produced according to claim 1,
25 having basal NTPase activity less than 150 min⁻¹ and
RNA helicase activity greater than 0.005 min⁻¹.

11. A process for preparing native, authentic,
enzymatically active NTPase/RNA helicase protein
comprising the steps of:

30 a) expressing an NTPase/RNA helicase
encoding nucleic acid of hepatitis virus
in a eukaryotic expression system such

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- 5 b) extracting said protein from said
 expression system, such that the
 extracted protein is in an enzymatically
 active form;
- 10 c) purifying said extracted protein from
 step b) such that the purified protein
 is an enzymatically active, native,
 full-length hepatitis C virus
 NTPase/RNA helicase protein.

15 15. A method for assaying a compound for anti-viral
activity against hepatitis C virus comprising:

- a) providing enzymatically active, native,
 authentic hepatitis C virus NTPase/helicase protein;
- b) contacting said protein with a compound
 suspected of inhibiting helicase activity; and
- 20 c) measuring inhibition of the helicase
activity in said protein by said compound.

 16. A method for assessing a compound for
anti-viral activity against a flavivirus, comprising:

- 25 a) providing enzymatically active, native,
 authentic flavivirus helicase protein;
- b) contacting said protein with a compound
 suspected of inhibiting helicase activity; and
- c) measuring inhibition of the helicase
- 30 activity in said protein by said compound.

 17. A method as claimed in claim 15, wherein
multiple compounds are assayed simultaneously.

 18. A method for assaying a compound for
anti-viral activity against hepatitis C virus

35 comprising;

- 5 a) providing an enzymatically active,
hepatitis C virus NTPase/RNA helicase protein;
- b) providing a partially duplex substrate in
which both strands are RNA and at least two nucleotides
at the 3' end of at least one RNA strand are not
10 involved in base pairing and at least one of said RNA
strands is detectably labeled;
- c) exposing said NTPase/RNA helicase protein
to said partially duplex RNA substrate in the presence
of a putative antiviral compound;
- 15 d) capturing any detectably labeled single
stranded release strand product of the interaction
between said RNA helicase protein and said substrate
with a capture system comprising a specific binding
pair, one member of said specific binding pair being
20 conjugated with an oligonucleotide having a nucleotide
sequence complementary to said detectably labeled
release strand and the other member of said specific
binding pair being affixed to a solid support; and
- e) quantitating detectable label present in
25 said release strand, as a measure of the anti-viral
activity of said compound.

19. A method according to claim 18, wherein
the other member of said specific binding pair is
30 affixed to a mobile solid support.

20. A method according to claim 18 in which
said oligonucleotide of said capture system is DNA.

21. A method according to claim 20 in which
35 said capture system comprises said oligonucleotide
conjugated with biotin and agarose beads coated with
streptavidin or a derivative thereof.

- 5 22. A method as claimed in claim 18, wherein multiple compounds are assayed simultaneously.

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